

# Detection of PPAR in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

## **Reagent and Antibody Information**

[1X Wash Buffer](#)  
[3% Hydrogen Peroxide](#)  
[1% BSA Diluent](#)  
[Carezyme II \(Pepsin\)](#)  
[DAB Chromagen](#)  
[Hematoxylin](#)

Blocking Serum: Normal Donkey Serum  
Jackson ImmunoResearch Laboratories, Inc.  
West Grove, PA 19390  
www.jacksonimmuno.com  
1-800-367-5296  
Catalog # 017-000-001

Avidin / Biotin Blocking Kit  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
www.vectorlabs.com  
1-800-227-6666  
Catalog # SP-2001

Primary Antibody: Rabbit Polyclonal To PPAR Antibody  
Abcam, Inc  
Cambridge, MA 02139  
www.abcam.com  
1-888-772-2226  
Catalog # ab8934

Negative Control Serum: Normal Rabbit Serum  
Jackson ImmunoResearch Laboratories, Inc.  
West Grove, PA 19390  
www.jacksonimmuno.com  
1-800-367-5296  
Catalog # 011-000-001

Secondary Antibody: Biotin-Conjugated Donkey Anti-Rabbit IgG  
Jackson ImmunoResearch Laboratories, Inc.  
West Grove, PA 19390  
www.jacksonimmuno.com  
1-800-367-5296  
Catalog # 711-065-152

Label Complex: R.T.U. Vectastain Elite ABC Reagent

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # PK-7100

**Staining Procedure**

Positive Control Tissue: Brown fat and mammary gland (pepsin); bile duct and salivary gland (EDTA)

Stain Localization: Cell membrane

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

4. Proteolytic-Induced Epitope Retrieval Using Pepsin

Preheat slides to 37°C in 1X Wash Buffer.

Incubate the slides in Carezyme II: Pepsin (predilute) for 8 minutes at 37°C.

(Allow the pepsin to reach room temperature before use.)

Rinse the slide in distilled water for 1 minute to stop the enzymatic reaction.

*(Note: May need to do decloaker / EDTA retrieval depending on the tissue type.)*

5. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

6. Block with 10% Normal Donkey Serum for 20 minutes at room temperature.

Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X Wash Buffer.

Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.  
ONLY WIPE EXCESS BLOCK.

8. Apply primary antibody at a 1:250 dilution. Incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_

For negative control slides, dilute the protein concentration of the normal rabbit serum to match that of the primary antibody. Make a 1:250 dilution from this normalized serum, and apply to the slides. Incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_

9. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

10. Apply the donkey anti-rabbit secondary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.

Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_

11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

12. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.

Exp. Date \_\_\_\_\_ New Kit: yes / no

13. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

14. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.

(Add 1 drop of DAB per ml of substrate)

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with Harris Hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X Wash Buffer until the tissues turn blue.

19. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip